

Genotypic Analysis of Multidrug-Resistant *Salmonella enterica* Serovar Typhi, Kenya

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We report the emergence in Kenya during 1997-1999 of typhoid fever due to *Salmonella enterica* serovar Typhi resistant to ampicillin, tetracycline, chloramphenicol, streptomycin, and cotrimoxazole. Genotyping by pulsed-field gel electrophoresis of *Xba*I-digested chromosomal DNA yielded a single cluster. The multidrug-resistant *S. Typhi* were related to earlier drug-susceptible isolates but were unrelated to multidrug-resistant isolates from Asia.

Salmonella enterica serovar Typhi causes approximately 10 million cases of typhoid that result in 600,000 deaths each year, mostly in developing countries (1). The antibiotics that form the mainstay of therapy in developing countries are chloramphenicol, ampicillin, and cotrimoxazole. Multidrug-resistant (MDR) strains of *S. Typhi* (resistant to all the above antimicrobial drugs) have caused outbreaks in the Indian subcontinent, Southeast Asia, and the Middle East since 1987 (2). Genetic studies have shown that resistance is encoded on an *HI1* incompatibility plasmid and is transferable (3). MDR *S. Typhi* has not caused problems in Africa, except in South Africa (4), nor in South and Central America (5), and most isolates have remained fully susceptible. During 1997-1999, a number of isolates of MDR *S. Typhi* were identified from patients with typhoid in Nairobi, Kenya. We have examined their genotypic relationship to each other, to sensitive strains from Nairobi, and to MDR *S. Typhi* from Southeast Asia.

The Study

We analyzed isolates of *S. Typhi* obtained in the Kenyatta National Hospital from blood cultures of 16 adults with typhoid from 1988 to 1993; from 22 cultures of 19 adults and 3 children from 1997 to 1999; and from 17 representative

MDR *S. Typhi* strains collected from 1990 to 1995 (6) from Pakistan (7), Hong Kong (4), Bangladesh (3), Kuwait (1), and India (1). We did not have access to isolates from 1994 to 1996, when no active surveillance was conducted. MICs of ampicillin, co-amoxyclov, cefuroxime, cotrimoxazole, chloramphenicol, gentamicin, streptomycin, tetracycline, nalidixic acid, and ciprofloxacin were determined by the E-test method (AB Biodisk, Solna, Sweden).

Macrorestricted (using *Xba*I) chromosomal DNA from the *S. Typhi* isolates was separated by pulsed-field gel electrophoresis (PFGE) with a CHEF DRII system (Bio-Rad Labs, Richmond, VA). The gels were stained with ethidium bromide and photographed on an ultraviolet transilluminator. Banding patterns were compared (8), and dendrograms of relatedness were constructed by data clustering using the unweighted pair grouping arithmetic averaging method (Molecular Fingerprinting Program version 1.4.1, BioRad). Conjugation experiments, plasmid extraction and electrophoresis, and incompatibility grouping were performed as described (7).

All 16 *S. Typhi* isolates from 1988-1993 were fully sensitive to all the drugs tested (MIC 0.012-0.016 mg/L for ciprofloxacin to 1-3 mg/L for chloramphenicol). In contrast, 18 (82%) of the 22 *S. Typhi* from 1997-1999 were resistant to ampicillin, tetracycline, and chloramphenicol (MICs all > 32 mg/L), as were the 17 isolates from Asia. The first two MDR *S. Typhi* from Kenya were detected from blood cultures from two

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adults in March 1997. Active surveillance is ongoing, and multidrug resistance is detected in approximately 65% of all *S. Typhi* isolates to date. As we did not have access to isolates from 1994-1996, we cannot be certain that MDR *S. Typhi* did not emerge earlier than 1997.

All the Kenyan MDR *S. Typhi* isolates came from indigenous patients with no known history of recent travel outside the country. The Kenyan MDR isolates remained sensitive to co-amoxycylav, cefuroxime, gentamicin, nalidixic acid, and ciprofloxacin. The Kenyan MDR *S. Typhi* all transferred their full resistance phenotype to *Escherichia coli* K12 on 98- to 100-MDa plasmids of inc HI1 (or inc HI1 cross-reacting with inc FIIA).

All 22 MDR and 16 sensitive *S. Typhi* were analyzed by PFGE. As all 22 MDR isolates were similar by PFGE, only two representative strains were selected for further analysis. In addition, 5 representative sensitive *S. Typhi* and 11 representative MDR strains from Asia were analyzed for similarity by using dendograms. The sensitive *S. Typhi* (1987-1992) had a number of different genotypes. The MDR *S. Typhi* were identical, but differed from the sensitive isolates by more than seven bands, indicating they were different strains. However, on the dendogram comparing MDR *S. Typhi* from Asia and the *S. Typhi* from Kenya (both MDR and sensitive), the Kenyan isolates formed one cluster, with the nearest (but genotypically quite distinct) other cluster being the Pakistani MDR *S. Typhi* (Figures 1 and 2).

Conclusions

The emergence of an MDR *S. Typhi* strain in Kenya is of concern because resistance to first-line antibiotics that are also commonly used for treatment of other bacterial infections in hospitals may pose a major challenge to health care. Although these newly emerged MDR *S. Typhi* are sensitive to nalidixic acid and ciprofloxacin, their MICs are five and ten times higher, respectively, than those of the sensitive *S. Typhi* from 1988-1993. Although fluoroquinolones are not widely available in Kenya, they may be needed to treat MDR *S. Typhi*, and resistance will lead to problems with treatment, as in Asia (9). Multidrug-resistant *S. Typhi* isolates from Kenya produced an indistinguishable PFGE pattern that was related to those of sensitive strains but unrelated

to those of MDR *S. Typhi* from Asia. This finding implies that the Kenyan MDR *S. Typhi* are most likely to have arisen from sensitive isolates by acquisition of resistance plasmids from antibiotic-resistant enteric bacteria. Plasmids of incompatibility group HI1 are those most frequently found in *S. Typhi*, but we did not detect them in any of our nontyphoidal salmonellae with the same plasmid-encoded resistance (7).

We observed the emergence of *S. Typhi* resistant to all first-line drugs used for treatment of typhoid in Kenya and in many other African

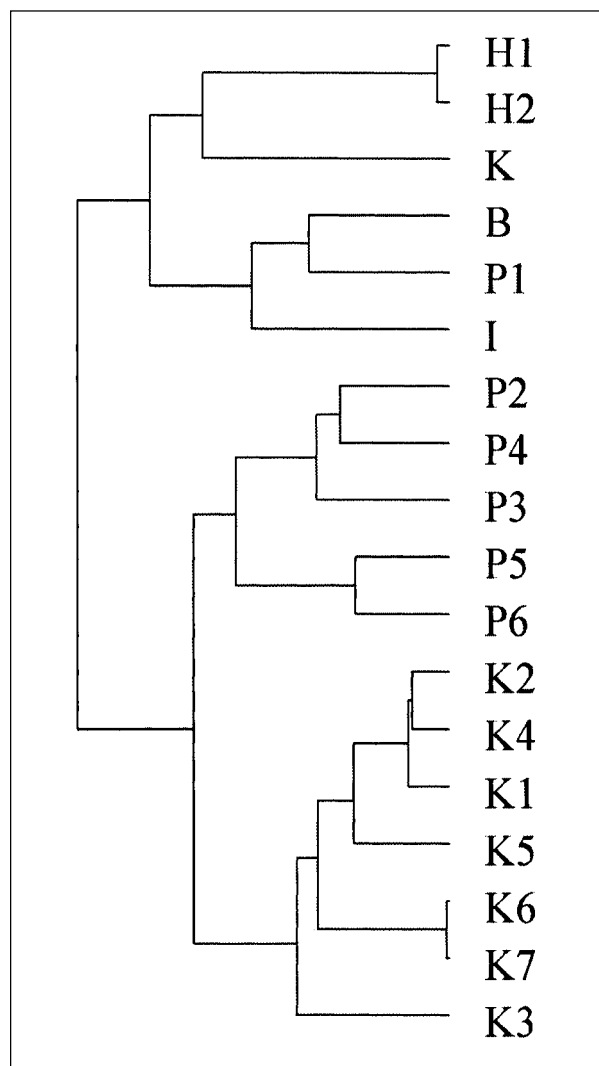


Figure 1. Dendrogram showing genetic relatedness of *Salmonella Typhi* from Kenya and Asia. H1 and H2: MDR *S. Typhi* from Hong Kong; K: MDR *S. Typhi* from Kuwait; B: MDR *S. Typhi* from Bangladesh; P1-P6: MDR *S. Typhi* from Pakistan, I: MDR *S. Typhi* from India. K1-K5: sensitive *S. Typhi* from Kenya; K6 and K7: MDR *S. Typhi* from Kenya.

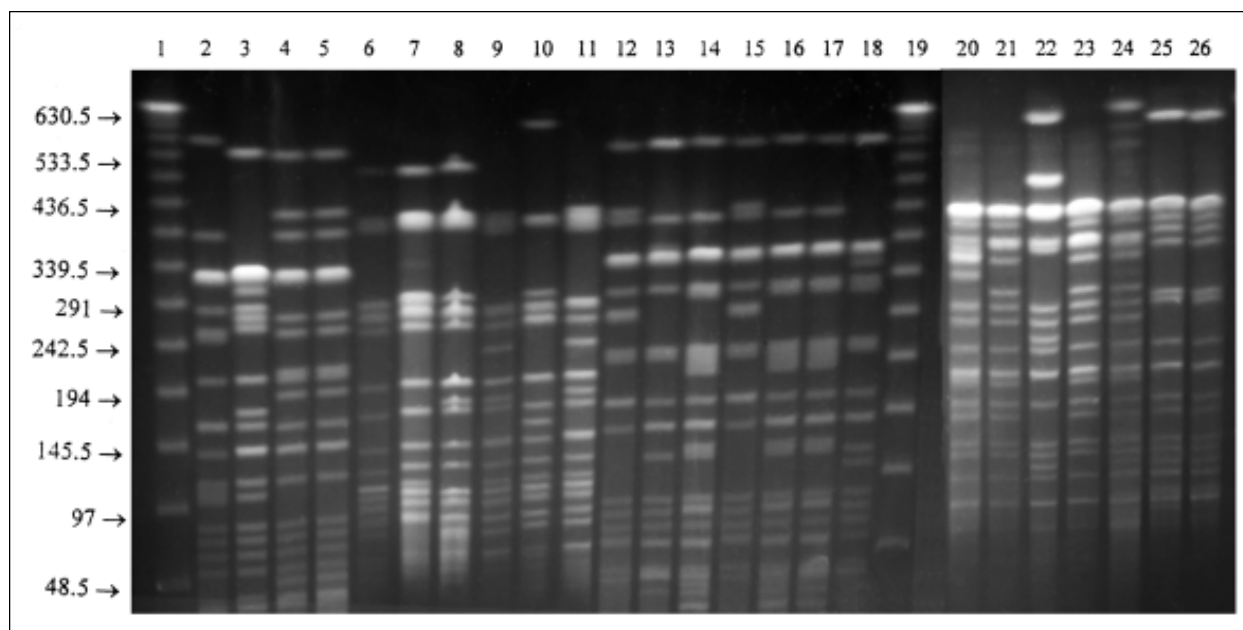


Figure 2. *Xba*I restriction endonuclease fragment patterns of representative *Salmonella* Typhi isolates from various countries. Lanes 1 and 19, molecular size standard; Lane 2, B1 from Bangladesh; Lane 3, I1 from India; Lanes 4 and 5, K1 and K2 from Kuwait; Lanes 6, 7, 8, and 9, M1, M2, M3, and M4 from Malaysia; Lanes 10, 11, 12, 13, and 14, Q1, A2, A3, A4, and A5 from Quetta; Lanes 15, 16, 17, and 18, R1, R2, R3, and R4 from Rawalpindi, Pakistan; Lanes 20-24, K1-K5: sensitive *S. Typhi* from Kenya; and Lanes 25 and 26, K6 and K7: multidrug-resistant *S. Typhi* from Kenya.

countries. Laboratories in Kenya should perform surveillance by routinely testing *S. Typhi* for susceptibility to first-line treatment drugs and to nalidixic acid to detect quinolone resistance. Effective surveillance for this newly emerged MDR *S. Typhi* in Africa and other developing regions of the world where MDR *S. Typhi* has not yet emerged would ensure prompt diagnosis, susceptibility testing, and appropriate antimicrobial chemotherapy.

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References

- Pang T, Levine MM, Ivanoff B, Wain J, Finlay BB. Typhoid fever: important issues still remain. *Trends Microbiol* 1998;6:131-3.
- Mirza SH, Beeching NJ, Hart CA. Multidrug resistant typhoid: a global problem. *J Med Microbiol* 1996;44:317-9.
- Shanahan PMA, Jesudason MV, Thomson CJ, Amyes SGB. Molecular analysis of and identification of antibiotic resistance genes in clinical isolates of *Salmonella typhi* from India. *J Clin Microbiol* 1998;36:1595-600.
- Coovadia YM, Gathiram V, Bhanjee A, Garratt RM, Mlisana K, Pillay N, et al. An outbreak of multiresistant *Salmonella typhi* in South Africa. *Quart J Med* 1992;82:91-100.
- Olarte J, Galindo E. *Salmonella typhi* resistant to chloramphenicol, ampicillin, and other antimicrobial agents: strains isolated during an extensive typhoid fever epidemic in Mexico. *Antimicrob Agents Chemother* 1973;4:597-601.
- Mirza S, Kariuki S, Mamun KZ, Beeching NJ, Hart CA. Analysis of plasmid and chromosomal DNA of multidrug-resistant *Salmonella enterica* Serovar Typhi from Asia. *J Clin Microbiol* 2000;38:1449-52.
- Kariuki S, Gilks C, Corkill J, Kimari J, Benea A, Waiyaki P, et al. Multidrug resistant non-typhi *Salmonellae* in Kenya. *J Antimicrob Chemother* 1996;38:425-34.
- Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33:2233-9.
- Murdoch DA, Banatvala NA, Bone A, Shoismatulloev BI, Ward LR, Threlfall JE. Epidemic of ciprofloxacin-resistant *Salmonella typhi* in Tajikistan. *Lancet* 1998;351:339.